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The rejection of claims 1-5, 11-21, 24-43, 45-66, 75-77, 79-80, 83, 87-88, and 138-148 under 35 U.S.C. § 103 for obviousness over Wiedmann, et. al., "Ligase Chain Reaction (LCR)—Overview and Applications," PCR Methods and Applications pp. S51-S64 (1994) ("Wiedmann") in view of Barany, "The Ligase Chain Reaction in a PCR World," PCR Methods and Applications pp. 5-16 (1991) ("Barany PCR"), U.S. Patent No. 5,415,839 to Zaun et. al. ("Zaun"), Guo, et. al, "Direct Fluorescence Analysis of Genetic Polymorphisms by Hybridization with Oligonucleotide Arrays on Glass Supports," Nucl. Acids Res. 22(24): 5456-65 (1994) ("Guo"), and U.S. Patent No. 5,648,213 to Reddy et al. ("Reddy") is respectfully traversed.

Wiedmann describes the use of the ligase chain reaction ("LCR") procedure to detect single base differences in target nucleic acids. LCR is disclosed to involve the use of 2 pairs of oligonucleotide probes. The first pair of probes is configured to hybridize to a first nucleic acid strand, while the second pair of probes is designed to hybridize to a second nucleic acid strand complementary to the first nucleic acid strand. The first and second pair of oligonucleotide probes are complementary to one another. As a result of using both pairs of oligonucleotide probes targeted to complementary nucleic acid strands, the LCR procedure is able to achieve exponential amplification. Wiedmann distinguishes the LCR procedure from the ligase detection reaction ("LDR") technique by virtue of the fact that LDR only uses a single pair of oligonucleotide probes which will hybridize to only one target nucleic acid strand and, thus, achieve linear amplification. There is no suggestion in this reference of detecting ligated products of an LDR procedure using a solid support, having capture probes which hybridize to an addressable array-specific portion of the ligated product as opposed to a distinct target-specific portion thereof. Instead, ligated products are separated by gel electrophoresis and detected by autoradiography or fluorescence.

Barany PCR is substantially the same as Wiedmann with the former being relied upon in the outstanding office action as teaching signal to noise ratios, various reporter groups, multiplex formats, detection of multiple mutations, operating conditions, and the use of thermostable ligases. Like Wiedmann, Barany PCR separates ligated products by gel electrophoresis and detects using autoradiography or fluorescence. Using LDR in conjunction with a solid support to capture ligated product sequences by hybridization of an addressable array-specific portion of the product which is distinct from the target-specific portion thereof is nowhere suggested by Barany.

Zaun discloses an apparatus and method for amplifying and detecting target nucleic acids. This procedure involves amplifying with a thermal cycling device and then detecting reaction products on a support having one or more reaction sites. Amplification can be carried out using the polymerase chain reaction ("PCR") or LCR procedures. To capture amplification products, the detection system is provided with a support having a plurality of capture sites to immobilize such products on the support. Zaun discloses capturing amplification products with antibody-antigen binding. The presence of captured nucleic acids is revealed by detection of a label bound to the nucleic acid with a specific binding pair. Such binding is preferably achieved with an antibody-antigen binding pair; however, chemical bonding and complementary polynucleotide procedures are also disclosed. See col. 14, lines 11-25 and col. 32, lines 21-53.

There is no enabling disclosure in Zaun of utilizing an LDR procedure for detecting single base changes, insertions, deletions, or translocations using oligonucleotide probe sets "configured so that the addressable array-specific portion is comprised of a nucleotide sequence which is distinct from that of the target-specific portions, in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence and the addressable array-specific portion", as set forth in claims 1 and 138. Support for this phrase is found, *inter alia*, on page 23, lines 13-20 of the present application. This feature permits the LDR procedure to be carried out under conditions which facilitate discrimination between target nucleotide sequences and other nucleic acids in a sample without effecting the subsequent detection procedure. See claim 150. Moreover, the sample can be subjected to a single set of ligation detection reaction conditions which facilitates more than one such reaction being carried out at a time. See claim 151. This is the case despite the fact that both the LDR and the solid support capture phases of the present invention involve hybridization to the oligonucleotide probe set. There is no suggestion of this aspect of the present invention in Zaun.

Zaun emphasizes the use of antigen-antibody binding to capture LCR products on a solid support. The problem with such binding is that different antigen-antibody binding pairs have differing levels of affinity, so a tremendous amount of experimentation would be needed to identify a sufficient number of different antigen-antibody binding pairs to capture and distinguish a large number of different LCR products on a solid support. By contrast, capture of ligated products by hybridization of an addressable array-specific portion thereof

to a capture probe on a solid support can be carried out by designing different addressable array-specific portions which hybridize to capture probes at similar conditions. The design of such a system is ascertained accurately and easily by matching reaction kinetics, largely by making T_m calculations. It thus can hardly be said that antigen-antibody binding is equivalent, let alone superior, to nucleic acid-nucleic acid hybridization as suggested by Zaun.

There is no mention in Zaun of designing capture oligonucleotide probes and addressable array-specific portions of sufficient length to achieve specificity, yet avoid cross-hybridization. This is because the preferred antibody-antigen binding of Zaun only requires consideration of these binding partners' relative affinity for one another; the concept of cross-hybridization to other binding partners is not considered. The failure to design capture oligonucleotide probes and addressable array-specific portions of proper length can lead to a false positive product signal being generated on the solid support regardless of whether there is target match or mismatch. Specifically, in the present invention, a match is detected when target-specific portions of the oligonucleotide probes hybridize to a target sequence, undergo ligation, and the ligated product is captured on a solid support by hybridization of an addressable array-specific portion to a capture probe. The second hybridization condition which captures the addressable array-specific portion on the solid support is sufficiently different from the hybridization condition during which ligation took place so that there is no carryover false signal through sandwich hybridization (i.e. hybridization to the capture probe of a first oligonucleotide probe which is bound to the target which is bound to a second oligonucleotide probe, where neither oligonucleotide probe is ligated together). Thus, in the present invention, a mismatch of the oligonucleotide probes when hybridized to a target sequence prevents ligation, and the two oligonucleotide probes dissociate from the target under the second hybridization conditions used to capture the addressable array-specific portion on the solid support.

To the extent Zaun mentions capture with complementary polynucleotides, there is no indication that this involves providing the oligonucleotide probes with an addressable array-specific portion which is distinct from the probes' target-specific portion. It is far more plausible that such polynucleotides instead hybridize to the target-specific portion of the probes. This presents great challenges in designing probes which can undergo both LDR and array hybridization, particularly where multiplexing is involved. The present

invention is a major advance over such a format in that the subject oligonucleotide probes can be designed to carry out LDR and solid support capture independently.

Guo relates to a multiplex PCR amplification procedure. This involves amplifying and detecting target nucleic acids using fluorescently labeled tags where the amplification products are denatured to render them single stranded, captured on a solid support with oligonucleotide probes, and detected. An important distinguishing feature of the present invention is that there is no need to denature ligated products to single stranded nucleic acid products; such products are either already single stranded or can be detected even if the ligated product is still hybridized to its corresponding target nucleic acid. Guo does not suggest using LDR at all, let alone in conjunction with a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide. In fact, Guo teaches away from the present invention by teaching that genetic polymorphisms can be detected by use of allele-specific oligonucleotides immobilized on glass slides.

Reddy relates to a composition and method for detection of analytes by using an assay system where one component is attached to a first member of a pair of complementary sequences forming a double stranded nucleic acid. The assay is carried out in the presence of a support to which the complementary nucleic acid is attached. Upon hybridization, the complementary nucleic acids hybridize to one another and are immobilized on the solid support. Immobilized analytes (bound to their respective antibodies) are then selectively displaced from the solid support, using a displacer oligonucleotide, with the analyte's presence in solution then measured by detection of labeled analyte. In particular, the portion of oligonucleotide sequence which hybridizes to the solid support has a length and T_m which gives the displacer oligonucleotide sufficient opportunity to displace the antibody-analyte complex from the solid support. In fact, were one to adapt the procedure of Reddy to the design of the addressable array-specific portion and the capture probes of the present invention, these oligonucleotides would only hybridize under conditions permitting false positive signal due to sandwich hybridization. This occurs because short complementary sequences are needed to achieve Reddy's displacement technique. Thus, Reddy teaches away from the present invention. Further, Reddy has nothing to do with LDR or the use of a solid support to capture ligated product sequences resulting from LDR by hybridization to a capture oligonucleotide. His technique is used to carry out immunochemical assays where the assay involves antibody-antigen or protein-protein binding where the target itself is

labeled. In the present invention, the presence of product is amplified and the existence of target is determined by detection of labeled ligation product as opposed to target itself. Even if one of ordinary skill in the art were to adapt Reddy's technique to LDR (which there is absolutely no suggestion of), he would configure the nucleic acid attached to the solid support to be complementary to the target specific portion of the ligated product; no addressable array-specific portion would be needed. As noted above with respect to Zaun, the present invention constitutes a major advance over such an arrangement.

As noted above, Weidmann, Barany PCR, and Zaun all relate to procedures involving LDR or LCR. On other hand, neither Guo nor Reddy involve LDR or LCR in any way whatsoever. Accordingly, one of ordinary skill in the art would have had no motivation, let alone ability, to adapt the teachings of Guo and Reddy to the LDR procedures of Weidmann, Barany PCR, and Zaun.

Even if the cited references were combinable, which they are not, their combination would not teach the claimed invention. As noted above, neither Weidmann, Barany PCR, Zaun, Guo, nor Reddy suggest adapting an LDR procedure to a subsequent solid phase capture procedure, as claimed. Claims 1 and 138 require oligonucleotide probe sets "configured so that the addressable array-specific portion is comprised of a nucleotide sequence which is distinct from that of the target-specific portions, in order to minimize hybridization between the target-specific portions and the capture oligonucleotides as well as between the target nucleotide sequence and the addressable array-specific portion". Although Zaun involves LCR and solid support capture, there is no suggestion of this feature. Zaun, at best, only suggests a solid support where solid capture involves hybridization to the target-specific portion of ligation products. This facilitates the procedure of the present invention where "sequences differing by one or more single-base changes, insertions, deletions, or translocations are discriminated from one another during the ligase detection reaction and the discriminated sequences are detected as a result of capture on the solid support" as set forth in claim 150. It also facilitates use of a "single set of ligase detection reaction conditions" pursuant to claim 151. Again, the combination of Weidmann, Barany PCR, Zaun, Guo, and Reddy provide no suggestion of any of these aspects of the present invention. Accordingly, the rejection based upon this combination of references should be withdrawn.

It is further submitted that this combination of references also fails to teach the parenthetical features of the following dependent claims: 4 (mismatch at a base adjacent to a

base at the ligation junction), 11 (quantifying target nucleotide sequences), 12, and 14-34 (detecting multiple allele differences), and 13 (target specific portions of oligonucleotide probes with substantially the same melting temperatures). Nowhere in the outstanding office action is it explained where these features are taught by the cited references. Therefore, the rejection of claims 4 and 11-34 based on the combination of Weidmann, Barany PCR, Zaun, Guo, and Reddy should be withdrawn.

The rejection of claims 6-10, 22, and 23 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, Reddy, and Telenti, et. al., "Competitive Polymerase Chain Reaction Using an Internal Standard: Application to the Quantitation of Viral DNA," J. Virol. Methods 39: 259-68 (1992)("Telenti") is respectfully traversed.

Telenti is cited as teaching that PCR can be quantitated by providing a known amount of an internal standard. However, this reference does not disclose the use of an internal standard in conjunction with an LDR process, nor does it involve the use of arrays. Therefore, Telenti does not overcome the above-noted deficiencies of Wiedmann, Barany PCR, Zaun, Guo, and Reddy.

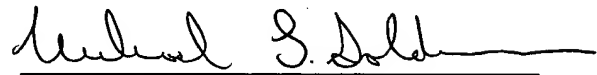
The rejection of claims 78, 82, and 84-86 under 35 U.S.C. § 103 for obviousness over Wiedmann in view of Barany PCR, Zaun, Guo, Reddy, and Sambrook, et. al., Molecular Cloning (1989)("Sambrook") is respectfully traversed.

Sambrook is cited for its teachings regarding hybridizing to immobilized nucleotides, barrier oligonucleotides, exonucleases to destroy nucleotides, and stripping blots. However, Sambrook does not involve the use of LDR in conjunction with arrays. In view of these deficiencies, the rejection based on the combination of Wiedmann, Barany PCR, Zaun, Guo, Reddy, and Sambrook should be withdrawn.

In view of all the foregoing, it is submitted that the present application is in condition for allowance and such allowance is earnestly solicited.

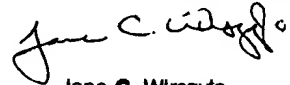
Respectfully submitted,

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Michael L. Goldman
Registration No. 30,727

NIXON PEABODY LLP
Clinton Square, P.O. Box 1051
Rochester, New York 14603
Telephone: (716) 263-1304
Facsimile: (716) 263-1600

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